GEL INSERTS USEFUL IN ELECTROPHORESIS

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This application is a continuation in part of U.S. Ser. No. 442,580, filed Nov. 18, 1982, now U.S. Pat. No. 4,473,452, issued Sept. 25, 1984, the contents of which are hereby incorporated by reference into this application.

BACKGROUND AND SUMMARY OF THE INVENTION

The invention is in the field of electrophoresis. It is of particular interest in terms of its application in genetic engineering and molecular biology.

Additional information pertinent to this invention may be found in Schwartz, D. C. and Cantor, C. R., "Separation of Yeast Chromosome-Sized DNAs by Pulsed Field Gradient Gel Electrophoresis," Cell, Volume 37, pg. 67, May 1984; and Van Der Pleog, H. T., Schwartz, D. C., Cantor, C. R. and Borst, P., "Antigenic Variation in *Trypanosoma brucei* Analyzed by Electrophoretic Separation of Chromosome-Sized DNA Molecules," Cell, Volume 37, pg. 77, May 1984.

The invention which is based upon the discovery of a new kind of electrophoresis makes it possible, inter alia, to carry out important analyses which were not possible or practical with previously known techniques. Potential applications include the separation of chromosomal DNA, chromosomal mapping, the convenient production of genetic libraries, studies on the effects of various drugs on chromosomal DNA, and the convenient characterization of polymers. The invention makes it possible to separate with a high degree of resolution and at high speeds larger particles (molecules) than those capable of resolution with prior art techniques and to concurrently separate particles which differ substantially in mass. In a preferred embodiment, the invention makes it possible to lyse cells for electrophoretic separation of macromolecules e.g. chromosomes contained within the cells with minimal degradation or breakage.

Electrophoresis in which particles such as a mixture of macromolecules are moved, e.g., through a gel matrix, by an electric field, is a widely used technique for qualitative analysis and for separation, recovery and purification. It is particularly important in the study of proteins, nucleic acids and chromosomes. See, e.g., Cantor, C. R. et al., *Biophysical Chemistry,* Freeman, 1980, Part 2, pp. 676, 683. Indeed, it is probably the principal tool used in most DNA and chromosomal analysis.

The particles to be analyzed and separated by electrophoresis are placed in a support medium such as a gel and are subjected to an electric potential. Difficulties arise when electrophoretic separation of very large particles is attempted. For example, using previously known techniques, the size of the largest DNA molecule routinely handled is that of a bacteriophage (3.2×10^7 daltons). Such a limit on size prevents many kinds of desirable analyses from being carried out. For example, intact chromosomal DNAs are larger and are typically reduced in size in order to make it possible to work with them. This, however, destroys important information encoded within the DNA and precludes many important experiments and analyses.

Methods of extending gel electrophoresis to particles of higher mass by reducing the gel concentrations have been proposed. However, this adversely affects resolution, makes experimental conditions difficult to control and has not been successfully applied to DNA molecules having molecular weight greater than about 5×10^8 daltons. Fangman, W. L., *Nucleic Acids Research,* Vol. 5, No. 3, March 1978, pp. 653–655; Serwer, P., et al., *Electrophoresis,* 1981, Walter, deGreuyter and Coe, pp. 237–243.

It is believed that resolution in previously known electrophoresis techniques is field-dependent since lower electric field intensities generally give higher resolution. As a consequence, electrophoresis runs in which higher resolution is desired often take as long as 100 hours. Moreover, particle mobility, and hence resolution capability, is believed to vary with the logarithm of the mass of the particles to be separated, which of course is not a highly sensitive basis for obtaining separations. Additionally, in known prior art gel electrophoresis, different gel concentrations are typically used for different mass or molecular weight ranges, thereby limiting the range of particles which can be concurrently resolved. Furthermore, previously known electrophoresis techniques are typically used to separate only small amounts of particles, and the process cannot conveniently be extended to larger amounts.

Another problem involved in the electrophoretic separation of large molecules e.g. DNA arises because the molecules (DNAs) must first be isolated since they may not exist as free molecules in the cell. For cells such as yeast and bacterial cells which have a cell wall isolation of DNA generally involves weakening the cell wall by treating it with an enzyme such as lysozyme for bacteria or zymolyase for yeast to form spheroplasts and with a chelating agent e.g. ethylenediaminetetraacetic acid (EDTA). For cells such as mammalian cells which do not have a well-defined cell wall it is of course not necessary to carry out such a treatment step. Cell lysis of spheroplasts or of cells which do not have a well-defined cell wall may be then accomplished by the addition of a detergent such as sodium dodecyl sulfate (SDS) in a buffered saline solution.

Following lysis, the solution is treated with pancreatic ribonuclease to hydrolyze RNA with protease to degrade proteins. Residual proteins and oligopeptides are extracted with an organic solvent, such as phenol or a mixture of equal volumes of phenol and chloroform. Most of the protein will denature and enter the organic phase or precipitate at the interface of the organic and aqueous phases. The clear, viscous aqueous phase containing the DNA may be removed. With the addition of alcohol, the DNA will precipitate out of the aqueous phase as a white fibrous material and may be spooled out on a glass rod. Precipitation from alcohol serves to concentrate the high molecular weight DNA while removing the small oligonucleotides of DNA and RNA, detergent and the organic solvent used in the removal of proteins. Residual detergent and salts may be removed by dialysis of the resuspended DNA solution against the desired buffer. In some instances, it may be desirable to further purify the DNA by centrifugation on isopycnic cesium chloride gradients or hydroxylapatite chromatography.

DNA molecules are extremely susceptible to breakage from shearing forces. As can be seen from the fore-